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HIPPOCAMPAL ESTRADIOL SYNTHESIS AND ITS SIGNIFICANCE FOR HIPPOCAMPAL SYNAPTIC STABILITY IN MALE AND FEMALE ANIMALS

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Abstract—Increasing evidence points to an essential role played by neuron-derived neurosteroids, such as estrogen, on synaptic connectivity in the hippocampus. Inhibition of local estradiol synthesis results in synapse loss specifically in females, but not in males. Synapse loss in females, after inhibition of estradiol synthesis in hippocampal neurons, appears to result from impairment of long-term potentiation (LTP) and dephosphorylation of cofilin, and thereby the destabilization of postsynaptic dendritic spines. Such clear-cut effects were not seen in males. Cognitive deficits after inhibition of aromatase, the final enzyme of estrogen synthesis, have been seen in women, but not in men. Altogether, the data demonstrate distinct differences between genders in neurosteroid-induced synaptic stability.

Key words: aromatase, estrogen, sexual dimorphism, synaptic plasticity.

Contents

INTRODUCTION

Steroids are essential for brain function, from the early steps of differentiation to the senescent brain during which they help to maintain neuronal performance and protect against damage. The brain is equipped with all enzymes active in steroidogenesis and is thus capable of synthesizing so-called ''neurosteroids'', which act locally to modify neural performance (Compagnone and Mellon, 2000; Shibuya et al., 2003). Steroids, however, also enter the brain from the peripheral circulation. Both pathways – paracrine and endocrine – are often interlinked. Thus, the precise attribution of the individual routes to physiological processes is not easily addressed.

As to the origin of steroids in the CNS, the varying density of spines along hippocampal dendrites of CA1 pyramidal neurons strongly supports the idea that estrogen of ovarian origin regulates spinogenesis in the hippocampus (Woolley et al., 1990; for review see Spencer et al., 2008). In addition, the removal of gonads has been shown to result in reduced dendritic spine density in this brain area of males and of females (Gould et al., 1990; Leranth et al., 2004). A rescue of spine loss after gonadectomy could be achieved by injections of estradiol in females but not in males. In gonadectomized males, injections of testosterone restored reduced spine density after removal of the testes (Leranth et al., 2004).

Our data from recent years point to hippocampus-derived estradiol as the main and primary player in estrogen-induced synaptic plasticity in females. We inhibited estradiol synthesis in hippocampal cultures (therefore in the absence of any other source of estradiol) either pharmacologically by using letrozole, which is an inhibitor of aromatase, the final enzyme of estradiol synthesis, or by knock-down of steroidogenic acute regulatory protein (StAR), the rate-limiting step in estrogen synthesis. Inhibition of aromatase in hippocampal slice cultures resulted in a significant reduction in the number of spine synapses in the CA1 hippocampal region (Kretz et al., 2004; Prange-Kiel et al., 2006). Similarly, knock-down of StAR, the protein that transports cholesterol through the mitochondrial membranes, where steroidogenesis commences, induced a downregulation of pre- and postsynaptic marker proteins (Fester et al., 2009). Systemic treatment of female mice with letrozole also significantly reduced spine synapse density and, most importantly, this effect was

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Abbreviations: fEPSP, field excitatory postsynaptic potential; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; LTP, long-term potentiation; StAR, steroidogenic acute regulatory protein; TBS, theta-burst stimulation.

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also seen in ovariectomized animals, thus after having removed the main source of estradiol in females (Zhou et al., 2010). The latter result strongly supports the notion that in females, estradiol originating from neurons is essential for synaptogenesis, rather than estrogen from peripheral sources (Prange-Kiel et al., 2013).

Nevertheless, we also found that estradiol synthesis in the female hippocampus is linked to the hypothalamic-hypophyseal axis via the regulatory role of gonadotropin-releasing hormone (GnRH) on hippocampal estrogen synthesis. We showed that spine synapse density increases dose-dependently upon GnRH stimulation in hippocampal cultures from female animals; this regulation functions via stimulation of aromatase. Thus, in females, brain sex steroid levels should correlate to circulating levels of sex hormones, which vary depending on the reproductive state of the organism throughout life (Prange-Kiel et al., 2008, 2013). This was recently confirmed by Kato et al. (2013), showing that in fact estradiol concentrations in hippocampal tissue correlate to estradiol concentrations in serum during the estrous cycle. Consistently, it was previously shown that cyclic peripheral concentrations of estradiol influence StAR expression in the brain (Meethal et al., 2009), thus estradiol concentrations in serum influence steroidogenesis in the brain, as the expression of StAR is the rate-limiting step in estradiol synthesis.

ESTROGEN SYNTHESIS AND SPINE SYNAPSE DENSITY IN THE HIPPOCAMPUS OF MALES AND FEMALES

Although it has been known for years that the brain is equipped with all enzymes active in steroidogenesis, our knowledge on differences in the capacity of neurosteroid synthesis between genders is fragmentary. Naftolin (1971) was the first to describe aromatase expression in the diencephalon in females. Aromatase is the final enzyme of estradiol synthesis and catalyzes the conversion of testosterone to estradiol. Ten years ago, we showed that the enzyme is actually functional. After a couple of days in culture, hippocampal neurons had secreted estradiol into the supernatant, which was determined by RIA (Prange-Kiel et al., 2003), which was confirmed shortly thereafter by Hojo et al. (2004). As to differences between genders, immunoreactivity of aromatase was seen in hippocampal neurons of male animals as well as in those of female animals. Laser scanning microscopy and subsequent image analysis did not reveal any difference in the expression of the protein between genders (Fester et al., 2012). Although protein expression does not allow for any conclusion on the activity of the enzyme, the amount of estradiol measured in the supernatant of cultivated ''female'' and ''male'' neurons was similar as well (Fester et al., 2012). In contrast to these *in vitro* findings we found clear-cut differences between genders, when we measured the content of estradiol in male and female hippocampal tissue by mass spectrometry. In hippocampal tissue of male animals and ovariectomized animals, the amount of estradiol was extremely low and below the sensitivity of our system. In contrast, in hippocampal tissue of females the amount of estradiol was easily measured. The differences between males and females in the amount of estradiol in hippocampal tissue corresponded to the differences between genders in estradiol concentrations in plasma (Fester, 2012).

Robust functional sex differences have been identified in the hypothalamic circuitry regulating reproductive function. Increasing levels of estradiol synthesized by granulosa cells in the ovaries and released into the blood stream trigger the release of GnRH from neurons scattered throughout the hypothalamus, which in turn induce the mid-cycle surge in luteinizing hormone (LH) that is essential for ovulation. In contrast, in male rats the pattern of LH release upon GnRH stimulation is tonic and acyclic, thus leading to a steady state of testosterone, which always exerts a negative feedback on GnRH release (for review see Gillies and McArthur, 2010). The lack of cyclic variations of GnRH/LH in males should result in differences between genders. In fact, we found that systemic inhibition of aromatase in mice had no effect on spine synapse density in males (Vierk et al., 2012). In males, an increase in synapse density was even found in tendency. In this context, it is of note that concentrations of testosterone, being the direct substrate for the enzyme aromatase, should increase in tissue as well as in serum after inhibition of aromatase activity. According to data by Leranth et al. (2004), showing that spine loss in response to gonadectomy was rescued by testosterone, aromatase inhibition in hippocampal neurons should actually result in an increase in synapse density in males. The failure of letrozole to increase spine density in males may be explained by insufficient activity of 5alpha reductase, which converts testosterone to dihydrotestosterone, the active metabolite with a higher affinity to androgen receptors. The role of hippocampus-derived testosterone in hippocampal cultures that originate from male animals are currently under investigation in our laboratory.

DOES SPINE SYNAPSE LOSS AFTER INHIBITION OF NEURONAL ESTRADIOL SYNTHESIS RESULT FROM THE EFFECTS OF ESTRADIOL ON THE SPINE CYTOSKELETON?

Both the function and stability of dendritic spines depend on an intact actin cytoskeleton (Honkura et al., 2008, Dent et al., 2010, Hotulainen and Hoogenraad, 2010). Our results suggest that aromatase activity in hippocampal neurons is required for the stabilization of postsynaptic spines. In stable spines, disassembly of F-actin is prevented as soon as cofilin, an actin-associated protein, becomes phosphorylated. In hippocampal slice cultures of female animals, we found pronounced dephosphorylation of cofilin, together with synapse loss, after 7 days of letrozole treatment. In mice treated with letrozole, we also found enhanced dephosphorylation of cofilin as early as after 6 h of treatment. Accordingly, intracellularly synthesized estradiol obviously induces phosphorylation of cofilin, presumably via activating LIM-1 kinase, as shown by Kramár et al. (2009b). Cofilin is itself regulated; it becomes inactive when phosphorylated by LIM-1 kinase,